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December 18, 2003

FAX

RECIPIENT/ PHONE NO.	FAX NO.	COMPANY/ CITY, STATE, COUNTRY
Examiner Badio	(703) 746-5003	U.S. Patent & Trademark Office

Robert E. Richards
FROM

18
PAGES (WITH COVER)

05213-0494

43170-279683
CLIENT/MATTER NO.

PLEASE CALL 404 815 6497 IF YOU HAVE DIFFICULTY WITH THIS TRANSMISSION.

COMMENTS

Inventor: D'AMATO ET AL.
Serial No. 09/780,650
Filed: February 12, 2001
For: ESTROGENIC COMPOUNDS AS ANTI-MITOTIC AGENTS

TO BE COMPLETED BY KS OPERATIONS CENTER

TRANSMISSION RECEIPT DATE/TIME: _____

COMPLETED BY: _____ JOB CODE 17853

#22½
m.m.
9/30/04

Patents

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
D'AMATO ET AL.	Examiner: **B. Badio, Ph.D.**
Serial No.: **09/780,650**	Art Unit: **1616**
Filed: **February 12, 2001**	
For: **ESTROGENIC COMPOUNDS AS ANTI-MITOTIC AGENTS**	

DECLARATION OF ROBERT J. D'AMATO UNDER 37 CFR §1.131

Robert J. D'Amato declares as follows:

1. I reside at Lexington, Massachusetts 02420, and am one of the inventors named in the above-referenced application for Letters Patent.

2. Attached hereto as Exhibit 1 are true and correct copies of several pages from my laboratory notebook. The dates of these pages have been redacted from the copies attached hereto. These notebook pages show tests conducted by me and demonstrate the ability of 2-methoxyestradiol to inhibit microtubule formation. All of the notebook pages comprising Exhibit 1 are dated prior to July 2, 1993.

3. Attached hereto as Exhibit 2 is a true and correct copy of a letter from me to Dr. Hamel with the National Cancer Institute. The date of this letter has been redacted from the copy attached hereto. The letter states as follows:

> This letter serves to formalize our conversation regarding the estrogen metabolite 2 methoxy estradiol which we have discovered is an effective chemotherapeutic agent and microtubule inhibitor.

CERTIFICATION OF FACSIMILE TRANSMISSION

I hereby certify that this paper is being facsimile transmitted to the Patent and Trademark Office (Fax No. 703-746-5003) on the date shown below.

Typed Name of Person Signing Certification:

Signature

December 18, 2003
Date

This letter (Exhibit 2) was written by me and is dated prior to July 2, 1993. This document has also been redacted to remove non-relevant material.

4. Attached hereto as Exhibit 3 is a true and correct copy of a letter from Christopher Dippel of Harvard Medical School to Mr. Takeda. The date of this letter has been redacted from the copy attached hereto. This letter states as follows:

> At the meeting, Dr. Folkman described the identification of previously unreported antiangiogenic properties of an known urinary metabolite of steroid metabolism, 2-methoxy-estradiol (2-MO). ***
>
> ***
>
> Upon returning to Boston, Dr. Folkman asked a postdoctoral fellow, Dr. Robert D'Amato to initiate the screening of 2-MOE for activity. Dr. D'Amato found that 2-MOE inhibits growth of vessels in the cam assay but not in the rabbit eye assay. In addition, he tested the compound in mouse against Lewis lung carcinoma and estimated a T/C of .35. In culture, 2-MOE was equally effective in preventing the growth of Lewis lung and endothelial cells, which suggests to Dr. D'Amato that 2-MOE is an antimitotic agent. Additionally, he has demonstrated that it inhibits microtubule formation similar to vinblastine, a current anticancer agent. When tested in vitro with AGM-1470, 2-MOE's effect was slightly increased.

This letter (Exhibit 3) was written by Mr. Dippel and is dated prior to July 2, 1993. This document has also been redacted to remove non-relevant material.

5. The foregoing documents clearly demonstrate that Dr. Folkman and I conceived of and actually reduced to practice the invention claimed in the above-referenced patent application prior to July 2, 1993.

6. The undersigned declares further that all statements made herein of his own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements, and the like so made, are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing on this application.

12/17/03
Date:

Robert J. D'Amato

[redacted]

Test: 2- methoxy estradiol (50 mg)

(1, 3, 5[10] Estratrien - 2, 3, 17β Triol 2- methyl ether)

Try 100 microgram/10ul on 6 eggs

100 microgram/10ul
+ 50 microgram/10ul β cyclodextrin tetradecasulfate

6 eggs

1,3,5(10) Estratrien-2,3,17β-Triol-2-methyl Ether

Sample I Estra alone at 100µg/10ul in 0.45% methyl cellulose

Sample II Estra ~~alone~~ at 100µg/10ul + BCD at 50µg/10ul (A0698-047-01)

6^30 a.m. Saturday Avg 1 = 38-40 hours.

Estra 100µg alone	(++) (++) (++) nice avascular zones (still some crystals)	
Estra 100µg/10ul BCD 50µg/10ul	(+) (++) still some crystals	EXHIBIT

1st steroid I have seen that inhibits by itself - Doesn't have ~~an~~ estrogen activity

British Biotech #94

Glass beads added to sample of 8.4mg - 168µl for 500µg/10µl plus 10µl Tween 80 = smooth suspension

30 am Saturday Aug 1 = 38 hours

BB94 500µg/10µl	Early polygons		
BB94 250µg/10µl	⊖		

BB94 96 hours - Monday

500 = ⊕ ⊖ ⊕ ⊕ ⊖ ⊕ ⊖

250 ⊕ ++ ++

9 30 am Monday Aug 3, = 96 hours ~~Estradiol~~

estradiol (++) (++) (++) - | Dead

crystals still + | ||

(+) (+) ⊖ ⊘ | |||

crystals still present

~~Does Not need → heparin or cyclodextrin~~

2-Methoxy estradiol continued to produce nice (2+) avascular zones in all eggs up to 6 days after implantation - zones fading on Day 7 - 8

This is most potent and long lasting angiogenesis inhib

[redacted]

For Lewis Leroy - Jeff Grossfeld = Asmira

BB94 suspended in PBS + 0.01% Tween80 (v/v)
at 2.5 mgm/ml + glass beads - shake overnight
Give each 20 gram mouse 0.24 ml (i.p.) daily
= 30 mgm/kg
= 25 mgm/10 ml | = 100 mgm = 40 ml
= 50 mgm/20 ml

2-methoxy-estradiol suspended in PBS + 0.01% Tween80
2 ml contains 50 milligrams + 0.2 methyl
= 25 mgm/ml
2.5 mgm / 0.1 ml per 20 gram mouse
S.C. / day
= 125 mg/kg

I put 150 mgm 2-methoxy-estradiol (Steraloids) into 6 ml 0.45% methylcellulose in Ringers - Sterilized thru Nalgene filter. Smashed Smashed large crystals & spatula. Added Rest sterilized glass beads. No methylene Tween80 → = 2.5 mgm/0.1 ml = 125 mg/kg
Rocked in cold room overnight

EXHIBIT 29

EN000348

138

3 Methoxy estradiol

200
① small zone ⊕
② small avascular zone ⊕
③ - floated off
④ - died

100
① small avascular zone ⊕
② small avascular zone ⊕
③ ⊖
④ ⊖

EM 12

400
① ⊖
② ⊖
③ ⊖
④ ⊖
floated off

200
① ⊖
② floated off
③
④

Estradiol

200 — floated off

100 — floated

Em 12

200 — floated

100 — floated

4 Hydroxy tamoxifen

200 — 2 died

100 — 2 died

140

(All compounds [illegible])

① 3 methoxy 2methoxy estradiol

- 200
  - ① float
  - ② float
  - ③ died
  - ④ float
- 100
  - ① faint effect
  - ② No effect
  - ③ died
  - ④ died.
- 50
  - ① floated
  - ② No effect
  - ③ died.
  - ④ No effect

② 4 methoxy estradiol

- 200
  - ① floated
  - ② floated
  - ③ No effect
  - ④ No effect
- 100
  - ① ⊖
  - ② ⊖
  - ③ ⊖
  - ④ ⊖

③ estrone

- 200 ⊖ all

④ 4 methoxy estriol

- 200
  - ① ⊖
  - ② died
  - ③ ⊕ good zone but edges of eg had mold
  - ④ ⊖
- 100
  - ① ⊖
  - ② ⊕
  - ③ ⊕
  - ④ ⊕
  - all 3 (±) ←

⑤ 3 carboxymethyl estradiol

- 200
  - ⊖
  - ⊕
  - ⊕
  - ⊕
  - all 3 ⊕ ←
- 100
  - ① died
  - ② faint effect
  - ③ died
  - ④ floated

⑥ 17 acetate estradiol

- 200
  - ① ⊖
  - ② faint effect
  - ③ floated
- 100
  - ① floated
  - ② ⊖

⑦ EM 12

- 400
  - ① (±)/⊕ ←
  - ② floated
- 200
  - ① ⊖
  - ② floated
  - ③ floated

⑧ 4 Hydroxy tamoxifen

- 200 ⊖
- 100 floated
- ⊖

Dr. D'Amato

(1) 3 methoxy-2-methoxy Estradiol 200µg and 100µg /10ul
(2) 4 methoxy-estriol - 200µg/10ul and 100µg /10ul
(3) Carboxymethyl-estradiol - 200µg/10ul and 100µg /10ul
(4) EM12 - 400µg/10ul and 200µg/10ul
(5) Colchicine - 100µg/10ul
200 Not done, to Brittle to handle
(8) 2 methoxy estrone - 200, 100, and 50µg/10ul

All insoluble, very hard to handle, many floated
to complete Aug 24th # 5, 6, 7

	200	– died floated faint affect	100µg	died died pellet broken apart (±)
2	200	– /pellet broken died faint	100µg	(±) died (+)
3	200	floated (+) died died	100µg	–/faint died (±)
4	400	– – died floated	200µg	– floated died floated
7	200	Not done Too Brittle	100	
8	200	(1) – (2) died (3) died (scar) (4) white spot c̄ avascular zone (±)	100	(1) died (2)(3) floated (4) small white spot with partial avascular zone (±)
8	50	died floated floated		

J. Folkman 148

Made new batch 2-Methoxyestradiol for Lewis Lung mice receiving AGM-1470

① To achieve dose of 125 mgm/Kg mouse,

② each 20 gram mouse must receive 2.5 mgm

③ If each 0.1 ml contains 1.25 mgm,

④ Then ~~[illegible]~~ a daily injection ~~will be~~ of 0.2 ml will administer 2.5 mgm

⑤ ~~To make~~

1.25 mg/0.1 ml = 12.5 mg/1 ml

⑥ So add 75 mgm to 6 ml = 12.5 mg/ml

⑦ Use old Ringers 0.45% methylcellulose (1989) and mixed c̄ Fresh Ringers 1:1 to make 0.22% methylcellulose

Put thru millipore

⑧ added heat sterilized glass beads

⑨ add 2 ul Tween 80

⑩ Put on shaker in old cold room.

at beginning - looks like fine particles but afterwards - like skim milk

[redacted]

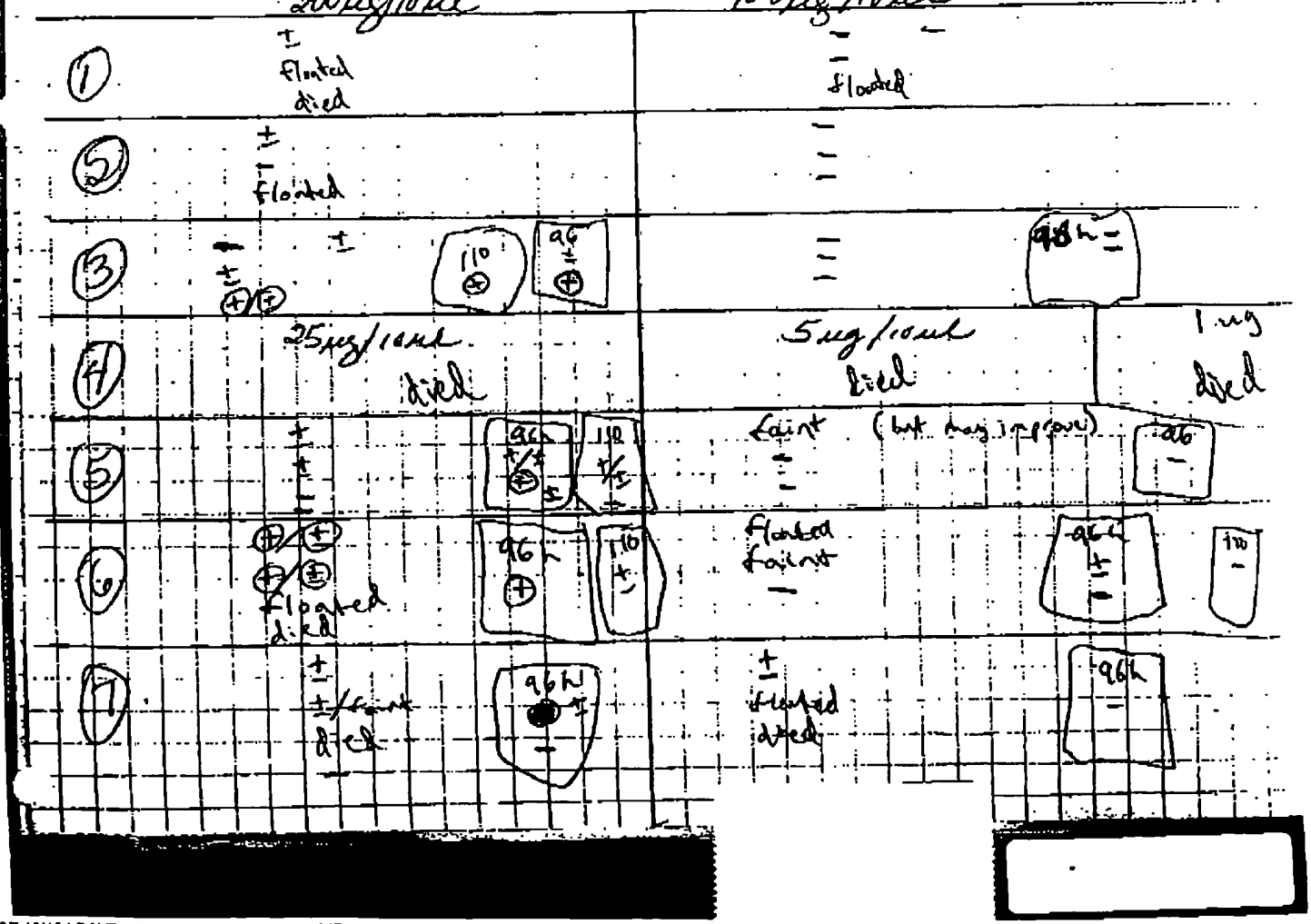
144

Dr. D'Amato

4 samples each concentration → 200 µg/10 ml
100 µg/10 ml

1. Estrone-3-Methyl ether
2. Estriol 3-Methyl ether
3. 2 Methoxy Estriol
4. Colchicine 25 µg and 5 µg
5. β-Estradiol Benzoate
6. 17 α Ethynyl estradiol
7. 17 α Ethynyl estradiol 3 Methyl Ether

#6 and #7 pieces - hard to handle



Dr. Watts

① Colchicine 1, 0.01, 0.1

② Cis-Retinoic Acid 200μg + 100μg, 10μg

③ 2-Methoxy-estradiol 100μg + 50μg

④ 3 Methoxy-2-Methoxy estradiol 200μg + 100μg

⑤ 4 Methoxyestriol 200μg + 100μg

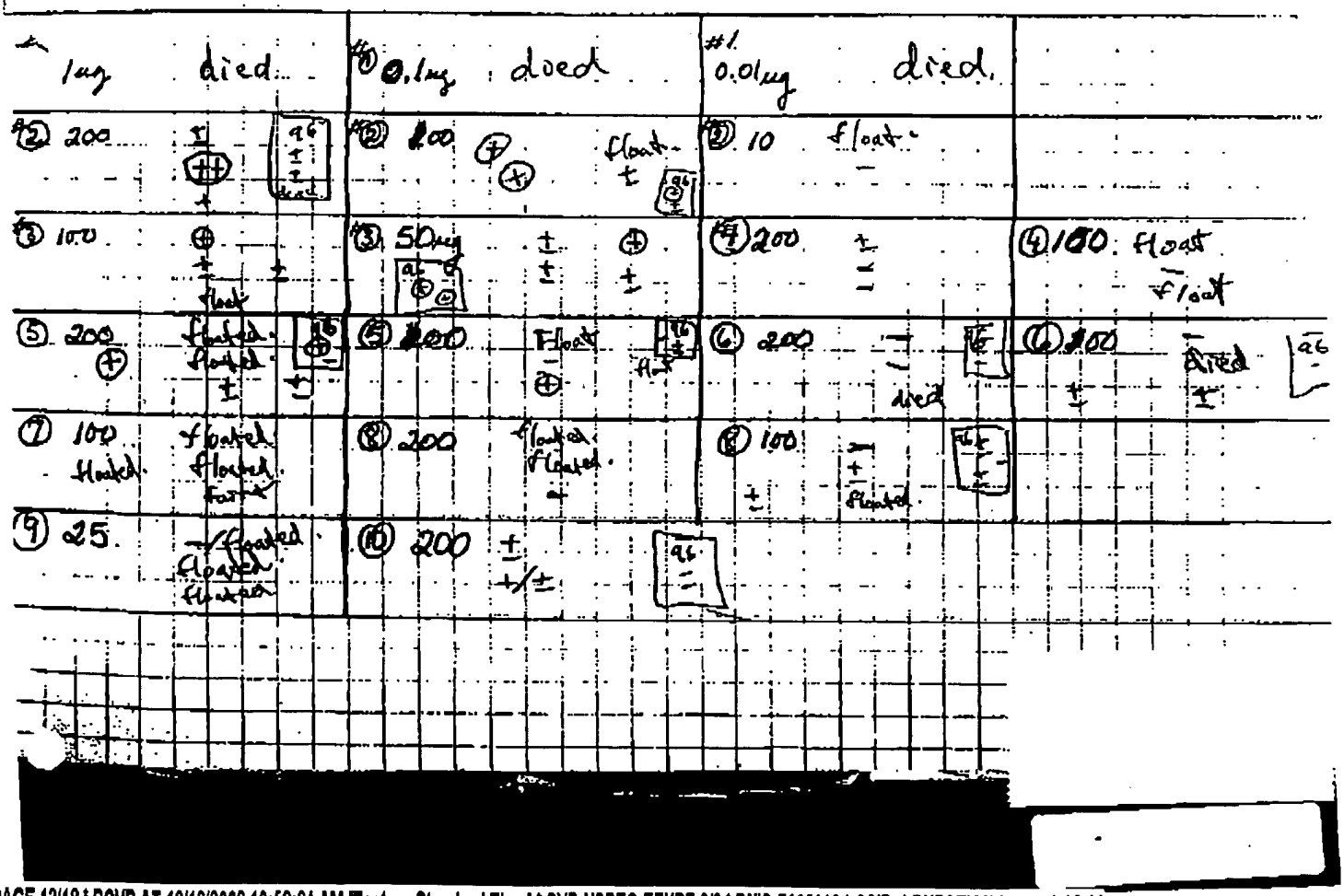
⑥ 3 Carboxymethyl Estradiol 200μg + 100μg

⑦ 2 Methoxyestriol 100μg

⑧ 17α Ethynyl estradiol 200 & 100

⑨ Nifedipine 25μg

⑩ Diphenylthiocarbazone 200μg



166

Vinblastin And Methyl Estradiol
on
12 DAY Gems

Vinblastin Concentration 0.025µg / 10µl → Zones of disrupted BI

Methoxye2 (Methoxy Estriol) — 24HOE

2MO - 100µg / 10µl

⊕ ⊕ ⊕ > small zones but real.

Robert D'Amato

1. 1, 3, 5 (10) Estratriene – 2, 3, 17 triol 2, 3, Dimethyl Ether
2. 1, 3, 5 (10) Estratrien 3, 4, 17, triol 4 methyl ether 200, 100, + 50
3. 1, 3, 5 (10) Estratrien 2, 3, 17 b 3 methyl ether
4. 2MOE 200µg 100µg

2 and # 3 at lower Concentration could not find dose 50µg, 25µg and 10µg

#1 200	± — DD —	#3 50	—
100	— — — D	#3 25	faint —
50	— — ± — —	#3 10	lots of crystals ⊖ — —
#2 200	— — — —	#4 200	— DD ⊕ ⊕ ⊕ F stools in cap
100	— — ± — D	#4 100	+/± +/± —
50	— — — D		
#3 200	— F D F		
100	— ⊕/± —		

HARVARD MEDICAL SCHOOL

DEPARTMENT OF SURGERY

THE CHILDREN'S HOSPITAL
300 LONGWOOD AVENUE
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(617) 735-7496 or 735-7497

Dr. Hamel
Building 37, Room 5c
NIH/NCI
Bethesda, MD 20892

Dear Dr. Hamel,

This letter serves to formalize our conversation regarding the estrogen metabolite 2 methoxy estradiol which we have discovered is an effective chemotherapeutic agent and microtubule inhibitor.

Sincerely,

Robert J. D'Amato

Robert D'Amato M.D. Ph.D.

Dept Surgical Research
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Boston, Mass 02115
617-735-6791
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EXHIBIT

Harvard Medical School

Office of Technology Licensing &
Industry-Sponsored Research

333 Longwood Avenue, Suite 640
Boston, Massachusetts 02115

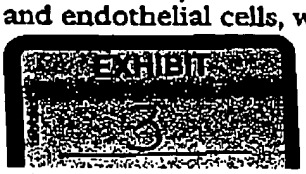
Telephone: 617-432-0920
Facsimile: 617-432-2788

Mr. Isao Takeda
Senior Manager, Licensing
Patent and Licensing Department
Takeda Chemical Industries, Ltd.
3-6, Doshomachi 2-chome
Chuo-ku, Osaka 541
JAPAN

Dear Mr. Takeda:

At the meeting, Dr. Folkman described the identification of previously unreported antiangiogenic properties of an known urinary metabolite of steroid metabolism, 2-methoxy-estradiol (2-MOE).

Upon returning to Boston, Dr. Folkman asked a postdoctoral fellow, Dr. Robert D'Amato to initiate the screening of 2-MOE for activity. Dr. D'Amato found that 2-MOE inhibits the growth of vessels in the CAM assay but not in the rabbit eye assay. In addition, he tested the compound in mouse against Lewis lung carcinoma and estimated a T/C of .35. In culture, 2-MOE was equally effective in preventing the growth of Lewis lung and endothelial cells, which suggests to Dr. D'Amato that



2-MOE is an antimitotic agent. Additionally, he has demonstrated that it inhibits microtubule formation similar to vinblastine, a current anticancer agent. When tested in vitro with AGM-1470, 2-MOE's effect was slightly increased. At this time, the results of these experiments have not been written up and Dr. D'Amato is now involved in other projects in addition to this one.

Sincerely,

Christopher Dippel
Project Manager

cc: Ms. Joyce Brinton
Mr. David Conlin
Dr. Robert D'Amato
Mr. William New
Dr. Judah Folkman
Ms. Carol Quilty